Title: Separation of Plant Pigments By Paper Chromatography

Introduction: Paper chromatography is one method that may be used to separate leaf pigments. The technique involves putting a spot or line of leaf extract on a piece of cellulose chromatography paper, allowing it to dry, and adding it to a solvent. The solvent moves up the paper by capillary action. This occurs as a result of the attraction of the solvent molecules to the paper and to each other. As the solvent moves up the paper, it carries along any substances dissolved in it, in this case, the plant pigments. The various pigments will be filtered out in bands or rings. The result is called a chromatogram.

The plant pigments are carried up the paper for different distances and at different rates because they are not equally soluble in the solvent and because they are attracted, to different degrees, to the cellulose in the paper through the formation of hydrogen bonds. The different molecular weights of each pigment also play a role in how high they climb up the paper.

Materials:
Chlorophyll solution (or fresh green leaf)
Solvent: acetone/petroleum ether (caution: fume alert!)
Glass jar or beaker with lid
Paperclips or staples
Quarter (25 cent piece)
Pencil
Metric ruler

Procedure:
1. Obtain a square of chromatography paper from your teacher. Use a pencil to draw a baseline exactly 1.5 cm from the bottom edge of the paper. Touch the paper as little as possible as dirt and skin oils can interfere with chromatogram development.
2. Place the paper, baseline facing up, on top of a fresh green leaf. Using the edge of a quarter, roll across the penciled baseline, applying moderate pressure. Be careful NOT to rip the paper! When you lift the paper, you should observe a dark green line of pigment on the reverse of the baseline. Allow the pigment line to dry completely before proceeding to the next step.
3. Place about 10 mL of chromatography solvent in a glass jar or beaker. Caution: avoid inhaling the solvent!
4. Form the paper into a cylinder, with the green pigment line at the bottom edge. Staple or clip each end so that the two edges are connected but do not overlap too much.

5. Cover the jar and do not disturb for several minutes.

6. Observe the separation of pigments until the solvent has climbed almost to the top of the cylinder. Complete Table 1. Since the solvent will evaporate rapidly and the pigments are often unstable and will fade or change color, you must work fast! As soon as you remove the cylinder from the jar, unroll it and flatten it on your desktop. Using a pencil, mark on the chromatogram the highest point to which the solvent flowed ("solvent front") and the highest peak to which EACH of the four pigments traveled.

7. Measure the distance (in cm, to the nearest tenth) each pigment traveled FROM THE BASELINE. Also measure the height to which the solvent traveled. Record this in Table 2.

Results and Observations:

1. Table 1: Plant Pigments

<table>
<thead>
<tr>
<th>Color band</th>
<th>Name of pigment</th>
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<tbody>
<tr>
<td>Dark yellow</td>
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<tr>
<td>Light yellow</td>
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<tr>
<td>Blue-green</td>
<td></td>
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<tr>
<td>Yellow-green (olive)</td>
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</table>

2. Draw a scaled-down color picture of your chromatogram. Label each pigment and distance measurement.
3. **Table 2: Relative distance traveled by Plant Pigments (Rf values)**

<table>
<thead>
<tr>
<th>Pigment name</th>
<th>Distance (cm)</th>
<th>Rf value</th>
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4. Show all **Calculations**. Be sure to label them by pigment.

Rf (retention factor) values reflect the relative differences between the various pigments. This is calculated by a simple division formula:

\[
Rf = \frac{\text{distance pigment migrated (from baseline)}}{\text{distance solvent front migrated from baseline}}
\]

**Conclusions and Discussion:**

1. How many color zones did you have on your chromatogram? List and describe them from top to bottom.

2. Are all of the color zones the same WIDTH (top to bottom)? Explain WHY this may be.
3. If you were to go outside and gather leaves from several different trees—What, if any, differences might you observe if this experiment was carried out with a variety of different types of leaves? Explain why!

4. What chemical factors are involved in the separation of pigments?

**Summary**: This may take some research!

5. What’s the POINT of having a variety of pigments in a leaf?

6. Explain the *changes* in coloration of leaves during various seasons of the year.